Recombination

- Homologous recombination
- Site specific recombination
- Models of homologous recombination:
    1. Holliday model
    2. Double strand break model

Recombination: A large scale rearrangement of a DNA molecule that involves the breakage and reunion of DNA

1st Recognized as the process responsible for crossing over during meiosis of eukaryotic cells and was subsequently implicated in the integration of the transferred DNA into bacterial genomes after conjugation, transduction or transformation

Types of Genetic Recombination

Homologous recombination
Exchange of homologous segments between any 2 homologous DNA molecules (or segments of same molecules) that share an extended homology

Site specific recombination
Recombination between two double stranded DNA molecule that have only short regions of nucleotide sequence similarity

Transposition: related to the processes of recombination, allows one DNA sequence to be inserted into another without relying on sequence homology

It provides a means by which certain elements move from one chromosomal location to another

Homologous recombination :

- It is also called general recombination
- Most important version of recombination in nature
- Responsible for meiotic crossing over in eukaryotes and the integration of acquired DNA by the process of conjugation, transduction and transformation into bacterial genomes
- It involves a reciprocal exchange of sequences of DNA

2 models that explains the Homologous recombination:

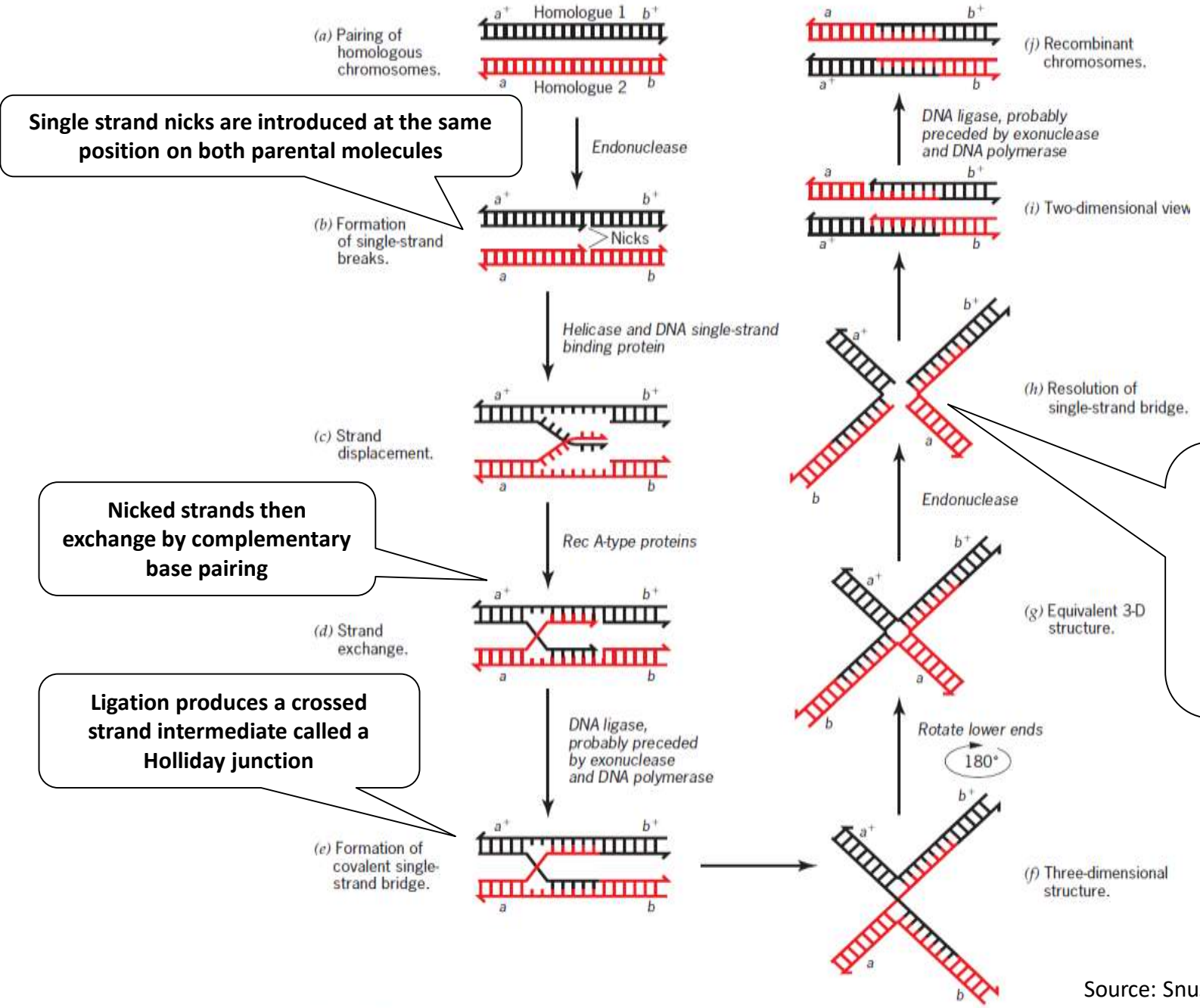
1. Holliday model
2. Double strand break model

Holliday model for homologous recombination

- An appealing scheme for homologous recombination was proposed by Robin Holliday in 1964
- This model is also known as heteroduplex model
- Describes recombination between two homologous double- stranded molecules, those with identical or nearly identical sequences
- But it is equally applicable to,
    - two different molecules that share a limited region of homology
    - to single molecule that recombines with itself because it contains two separate regions that is homologous with one another

Features of the Holliday model

- Formation of heteroduplex resulting from the exchange of polynucleotide segments between the two homologous molecules
- Heteroduplex is stabilized by base-pairing between each transferred strand and the intact polynucleotide of the recipient molecule
- Gaps are sealed by DNA ligase, giving a Holliday structure
- Holliday structure: dynamic, on branch migration resulting in exchange of longer segments of DNA, if the two helices rotate in the same direction



Single strand nicks are introduced at the same position on both parental molecules

Nicked strands then exchange by complementary base pairing

Ligation produces a crossed strand intermediate called a Holliday junction

Separation/ resolution of Holliday structure back to individual double stranded molecules occurs by cleavage across the branch point

■ FIGURE 13.29 A mechanism for recombination between homologous DNA molecules. The pathway shown is based on the model originally proposed by Robin Holliday in 1964.

Source: Snustad, Simmons (2012), Principles of genetics, John Wiley& Sons, Inc, Sixth edition, Page no. 355

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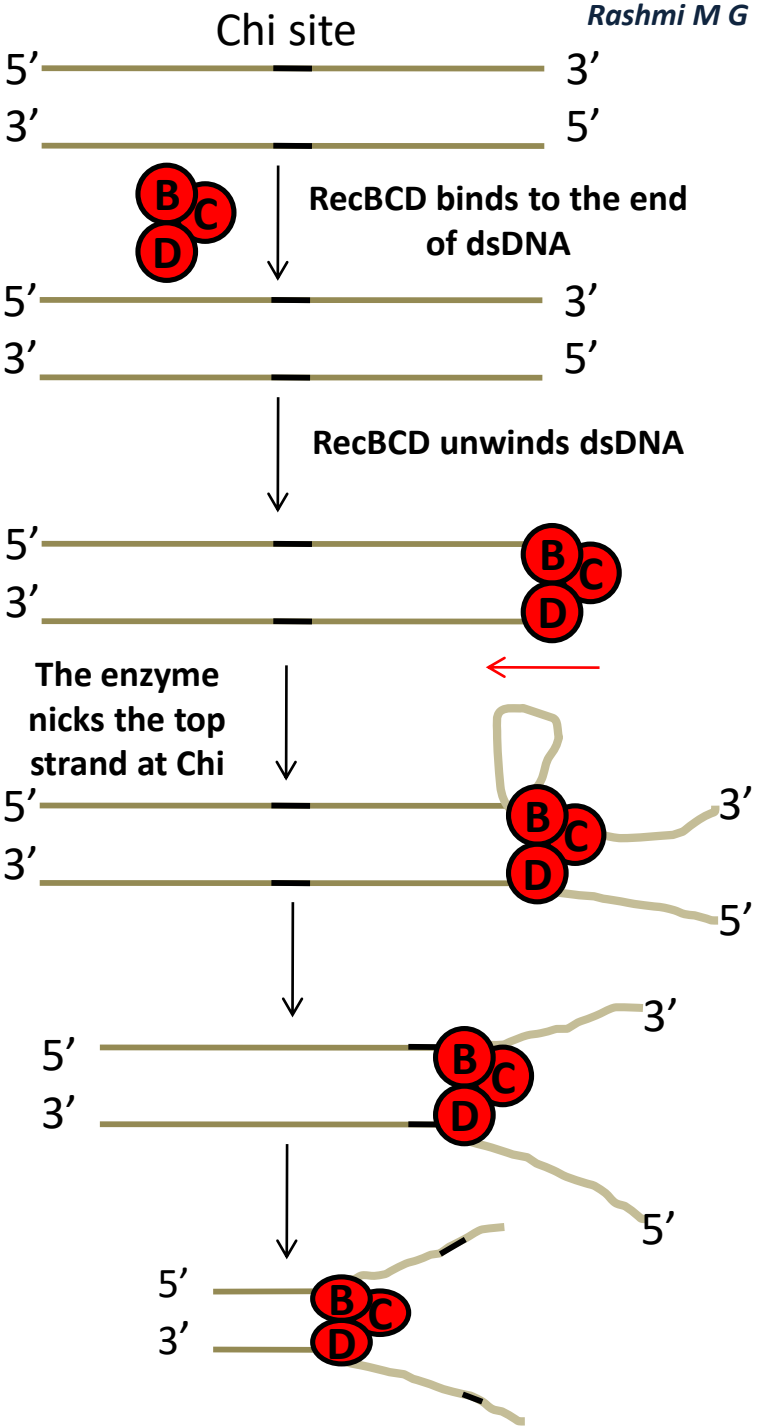
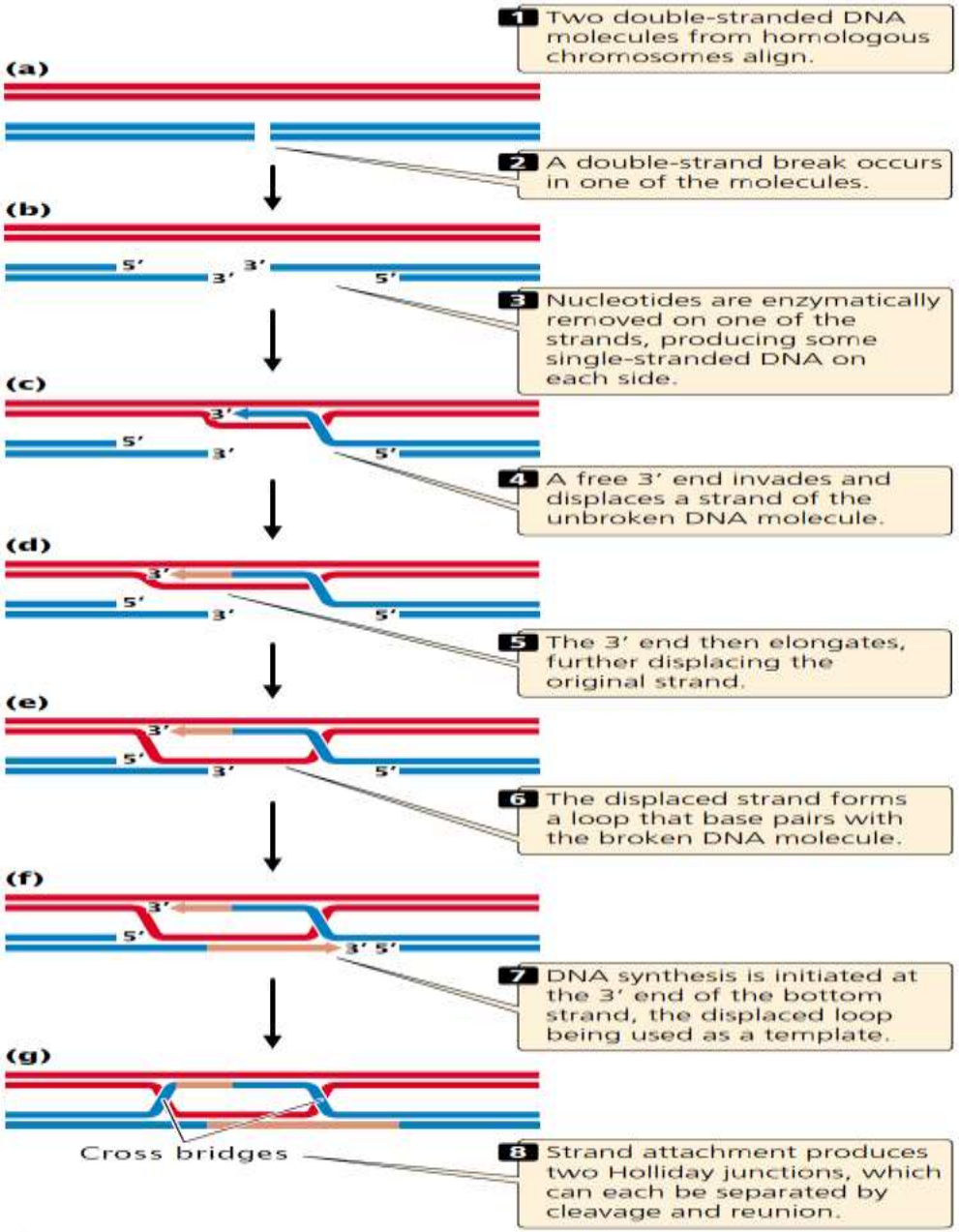
Proteins involved in homologous recombination in *E coli*

- 3 different recombination systems-
  - RecBCD (Most important)
  - RecE
  - RecF
- Recombination is initiated by RecBCD enzyme (Known as Exonuclease V) has both nuclease and helicase activities
- Rec BCD- is a bipolar helicase composed of 3 different subunits
  - Rec B- ATP dependent 3'-5' helicase activity, Nuclease activity, travels on one strand in 3'→5' direction
  - Rec C- recognizes a specific sequence in DNA known as *chi*
  - Rec D- ATP dependent 5'-3' helicase activity and nuclease activity, travels on other strand in 5'→3'direction
- RecBCD binds to linear DNA at a free (broken) end and moves inward along the double helix, unwinding it
- Rec D helicase travels on the strand with a 5' end at which the enzyme initiates unwinding, Rec B on the strand with a 3' end
- Activity of the enzyme is altered when it interacts with chi (Crossover Hotspot Instigator) sequence
- When RecBCD enzyme reaches the chi site, Rec C recognizes Chi site and signals Rec D to stop unwinding DNA
- Rec D then signals Rec B to cut DNA
- Rec B nicks the strand with chi (the strand with the initial 3' end)
- Unwinding continues and produces a 3' strand tail with chi at its terminus



Double stranded break model

- Initiation is by double strands break in one of the two DNAs
- DNA is resected from the breaking point by an exonuclease that leaves 3'-OH overhangs – DNA resection
- One of the overhang invades the homologous region in the other (donor) duplex – single strand invasion
- Formation of heteroduplex DNA generates a D loop, in which one strand of the donor duplex is displaced


(a)
1 Two double-stranded DNA molecules from homologous chromosomes align.
2 A double-strand break occurs in one of the molecules.
(b)
5′ 3′ 3′ 5′
3 Nucleotides are enzymatically removed on one of the strands, producing some single-stranded DNA on each side.
(c)
3′ 5′ 3′ 5′
4 A free 3′ end invades and displaces a strand of the unbroken DNA molecule.
(d)
3′ 5′ 3′ 5′
5 The 3′ end then elongates, further displacing the original strand.
(e)
3′ 5′ 3′ 5′
6 The displaced strand forms a loop that base pairs with the broken DNA molecule.
(f)
3′ 5′ 3′ 5′
7 DNA synthesis is initiated at the 3′ end of the bottom strand, the displaced loop being used as a template.
(g)
Cross bridges
8 Strand attachment produces two Holliday junctions, which can each be separated by cleavage and reunion.

12.24 **In the double-strand-break model, recombination is accomplished through a double-strand break in one DNA duplex, strand displacement, DNA synthesis, and resolution of two Holliday junctions.**

Source: Benjamin A Pierce, Genetics, A conceptual approach, Page no. 347

Site specific recombination :

- Process initiated between two DNA molecules that have only very short sequences in common
- Integration of bacteriophage ʎ DNA into the *E coli* genome involves site specific recombination
- Integration occurs by site specific recombination between the att sites, one on the ʎ genome and another on the *E coli* chromosome, which have at their center an identical 15-bp sequence
- Bacterial attachment site = att B (23bp) consisting of the sequence components BOB'. The B and B' are made up of 4 bp each
- Phage attachment site= att P (250 bp) consisting of the sequence components POP'
- The sequence O is common to att B and att P. It is called the core sequence and the recombination event occurs within it
- Recombination catalyzed by ʎ integrase (ʎ Int) (this enzyme use a conserved tyrosine residue to break phosphodiester bonds and covalently attaches to the 3'- phosphoryl group
- Integration also requires integration host factor (IHF)
- **Function of IHF** - to bind to sequence of ~20bps in att P and bring together the ʎ integrase binding sites on the DNA arms

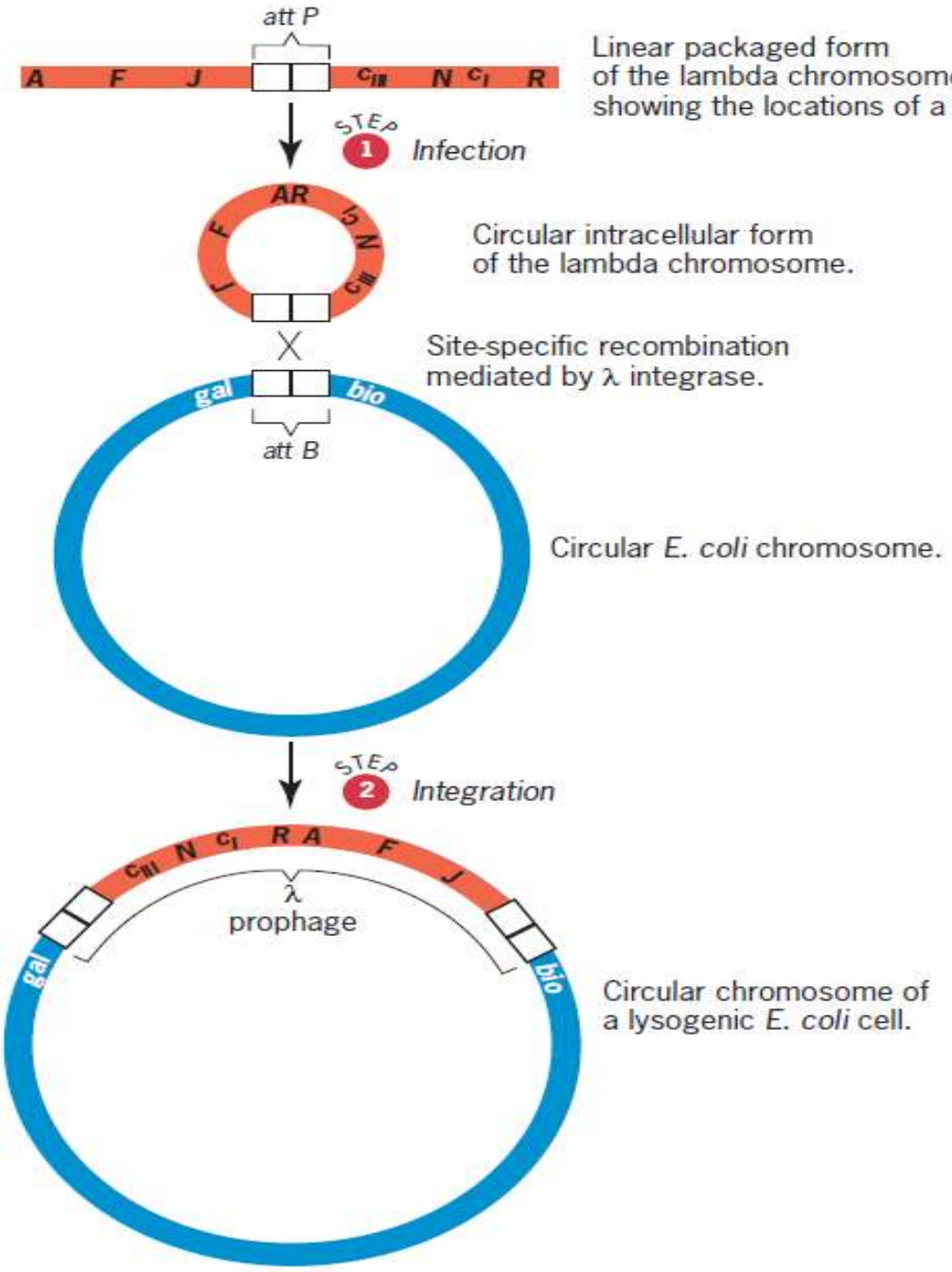

att P
A F J cIII N cI R
Linear packaged form of the lambda chromosome showing the locations of a few genes.
STEP 1 Infection
AR
J F N cIII
Circular intracellular form of the lambda chromosome.
Site-specific recombination mediated by λ integrase.
gal
bio
att B
Circular E. coli chromosome.
STEP 2 Integration
cIII N cI R A F J
λ prophage
gal
bio
Circular chromosome of a lysogenic E. coli cell.

FIGURE 8.5 Integration of the λ DNA molecule into the chromosome of *E. coli*.

- Both lambda and *E coli* DNA have a copy of the att site, each one comprising an identical central sequence called 'O' and flanking sequences P and P' (for phage att site) or Band B' (for bacterial att site)
- Recombination between the O regions integrates the lambda genome into bacterial DNA

Source: Snustad, Simmons (2012), Principles of genetics, John Wiley& Sons, Inc, Sixth edition, Page no. 169